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IN VITRO INHIBITORY ACTIVITY OF WEEDS EXTRACTS AGAINST *Macrophomina phaseolina* ASSOCIATED WITH CHARCOAL ROT OF *Sesamum indicum*

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ABSTRACT

Antifungal activity of aqueous extracts of above ground parts (leaves + shoots) of eight weeds Ageratum conyzoides L., Parthenium hysterophorus L., Datura metel L. Convolvulus arvensis L., Calotropis procera Aiton., Agremone Mexicana L., Senna occidentalis L. and Amaranthus viridis L. were examined against Macrophomina phaseolina. Fungicidal activity of test extracts was measured on growth and sclerotial development at 1, 2 and 3% dose levels by using food poison technique. All test weed species except P. hysterophorus showed maximum inhibition activity at their highest (3%) concentration. Datura metel exhibited 75.9% inhibition at 3% extract, followed by C. procera (63%) and C. arvensis (60%). Least inhibition of 11.2% was noticed in A. viridis treatments whereas intermediate growth inhibition ranging from 20-41% was exhibited by higher (3%) concentration of S. occidentalis, A. Mexicana and A. conzydus. Moreover, no sclerotial production was observed at all extracts concentarion of C. procera, D. metel and P. hysterophorus. While A. viridis, S. occidentalis, A. Mexicana, C. arvenis and A. conyzoides extract exhibited 29-70% reduction in number and 23-63% in size of sclerotia at 3% concentration. Present investigation concluded that weeds can be utilized for the management of M. phaseolina which caused charcoal rot of S. indicum.

Key words: Antifungal activity, aqueous extract, growth rate, *Macrophomina phaseolina, Sesamum indicum, Datura metel.*

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INTRODUCTION

Sesame, an important conventional and medicinal oil seed crop, is a rich source of protein fat, minerals, carbohydrate and vitamins. It is cultivated in a wide range of atmospheres, extending from semi-arid tropics and subtropics to temperate areas of the world (Raikwar and Srivastva, 2013). Major problem of sesame is its high susceptibility to a wide range of fungal diseases. Among which charcoal rot caused by *Macrophomina phaseolina* is most damaging and resulted in poor seedling development and 5-100% yield loss of the crop (Aly *et al.,* 2006). The fungus survives in soil for several years as saprophyte and has the ability to defuse plant, animal, as well as human immunity in immune-suppressed patients (Arora *et al.,* 2011). Complex pathogenic behavior and biochemical constituents of *M. phaseolina* have made its management a difficult task for the farmers (El-Bramawy1 and El-Sarag, 2012). In Punjab, farmers generally apply Topsin M70 and Carbendazim due to easy availability but it is an expensive and hazardous strategy. Nowadays demand of synthetic fungicide is reduced because of their non-biodegradable and pollutive nature. Moreover, residual toxicities of chemical fungicides cause irritation and diseases in humans and farm animals (Brindha *et al.,* 2009). Among the various types of disease strategies applied for crop protection, utilization of phyto extracts is comparatively safe and management effective. Weeds which are aggressive growers and successful competitors with crops possess fungicidal potential because of their active compounds (Prakash *et al.,* 2012). Earlier many scientists used extracts of *P. hysterophorus, Lantana camara*, *Ageratum conzoides, Datura metel, Xanthium strumarium* against *Alternaria alternate* and *Fusarium oxysporum* (Srivastava and Singh, 2011). While *Calotropis gigantia* and *Euphorbia antiquorum* were found inhibitory for the growth of post-harvest fungi i.e. *Aspergillus flavus, A.niger, Botryodiplodia theobromae* and *Penicillium chrysogenum* (Rajmane and Korekar, 2012).

In the present investigation, attempts are made to explore fungi toxic potential of abundantly available weeds during the growing season of the crop.

MATERIALS AND METHODS

Isolation of test pathogen

Isolate of *M. phaseolina* (accession no. 1156 FCPB) was isolated from sesame plant showing characteristic symptoms of charcoal rot. The affected portions of the plants were surface sterilized with 1% NaOCl solution to remove surface contaminants, washed with sterilize distilled water and placed in 90 mm Petri plate containing potato dextrose agar (PDA) medium. The plates were incubated in dark at

28±2 °C. Pure culture of isolate was maintained on potato dextrose agar in refrigerator at 4 °C.

Collection of plant material

Fresh Plant materials comprising above ground parts of all the test weed viz. *A. conzydus, C. arvensis, D. metel, A. viridis, P. hysterophorus, S. occidentalis, A. Mexicana* and C*. procera* were collected from field and wasteland areas of suburbs sites of Lahore district of Punjab Pakistan. Before processing weeds were identified on the basis of plant taxonomy. Stock plants material was rinsed under running tap water for 2-3 times for removal of soil particles attached as dust or dirt and initial contaminants. After washing plants were placed on strainer for 2-3 hrs for draining of excessive water. Plants were disinfected with 1% NaOCl, washed with distilled water, and shade dried on blotter paper. Dried plants were powdered with the help of electrical household grinder at medium revolution.

Preparation of plant extractions

Twenty grams powdered plants were soaked in 100 ml of sterilized distilled water for 24 h at room temperature. Aqueous extract was prepared by filtering soaked material through double layered muslin cloth and finally through Whattman filter paper No. 1 under sterilize condition (Chaudhary and Tariq, 2006).) The filtrate was considered as stock extraction with (20%) concentration.

Antifungal assay

The inhibitory effect of phytoextracts of test weed species against *M. phaseolina* was evaluated *in vitro* conditions by applying food poison technique. Extract concentrations (1-3%) of test plants were incorporated into potato dextrose agar media approximately at 40^{o}Cafter autoclaving at 121^{o}C and 1.035 × 10^{5} Pa pressure for 30 min. Plain agar media without the amendment of plant extract was used as control treatment. Media was poured in Petriplates and inoculation was done by placing 5mm mycelia disc from periphery of 7 days old culture of test fungi at the center of Petriplate with the help of cork borer. The test treatments were kept in incubator at 28±2°C.

Data collection

The experiment was performed in Completely Randomized Design withfivereplicates. Data for colony diameter and sclerotia production was recorded at 24hr interval. Percentage growth inhibition was measured by formula given below. No of sclerotia per microscopic field was counted from five replication of each treatment. The sclerotial formation was grouped on the basis of following score (Table-1) (Das and Tendal *et al.,* 2010). Percentage inhibition in sclerotia size was also recorded for each treatment.

$$\text{Percent inhibition (\%)} = \frac{\text{Radial growth in control - radial growth in treatment}}{\text{Radial growth in control}} \times 100$$

Table-1. Scale for number of sclerotia per microscopic field

Score	Level of sclerotia formation
0	No seclerotia formation
10-20	Low level
21-30	Medium level
< 30	High level

Statistical Analysis

Statistical analysis of the data was carried out using DSTAT. Inhibition in mycelial growth due to weeds extracts was subjected to analysis of variance (ANOVA) and the significance of the difference between means of treatments were compared with Duncan multiple range test at 5% level of significance.

RESULTS AND DISCUSSION

Sensitivity of aqueous extracts of test population of weeds viz *A. conyzoides,C. arvensis, D. metel, A. viridis, P. hysterophorus, S. occidentalis, A. Mexicana* and *C. procera* was examined against *M. phaseolina.* The intensity and nature of effectiveness was examined on the basis of radial growth (mm) and sclerotia production with respect to number and size. The quantitative description of observations explained the wide range of variation in mode of effectiveness in delaying colony growth and sclerotia formation. The highest reduction of 75% in radial growth was recorded in *D. metel* and least 11.2% was observed in *A. viridis*. Whereas 20-41% reduction in radial growth was induced by *A. mexicana, S. occidentalis, P. hysterophorus* and *A. conyzoides* (Fig. 1).The dose effectiveness analysis among the test concentration expressed strong relationship between the dose and increased reduction of colony growth. However *P. hysterophorus* presented a deviating trend from the generalized inhibitory effect. In this case on increasing the dose from 1 to 3%, an increase in radial growth from 53.8-62.2 mm was observed (Fig. 2). Inhibition in fungal growth at lower concentrations of aqueous extract was observed earlier as 2% of both root and shoots extract of *P. hysterophorus* significantly suppressed the biomass of *M. phaseolina.* Similarly *P. hysterophorus* also displayed maximum inhibition against *D. hawaiiensis* at lower concentrations (Bajwa *et al.,* 2007).

Development of sclerotia is typical characteristics of *M. phaseolina* which generally starts appearing after 48 hrs. Due to sclerotia formation fungus can survive more than five years even in the absence of suitable host. Therefore weed extracts were also tested for their inhibitory effect on sclerotia size (µm) and count (Table-1; Tandel *et al.,* 2010). The trend of sporulation and sclerotia formation was found in link with radial growth in case of *D. metel* and *C. procera*

as complete inhibition (100%) of sclerotia formation was recorded at all tested concentrations. However, inhibiting trends of *P. hysterophorus* against sclerotia formation varied than radial growth as it completely inhibited sclerotia formation at all test doses. They are followed by *A. conyzoides* and *C. arvensis* with 70 to 61% inhibition in number of sclerotia and 63.8 to 57.8% reduction in size whereas *A. viridis, S. occidentalis* and *A. Mexicana* induced (29-53%) reduction in number and (23-51%) in size respectively (Table-2).

Among test plants, *D. metel* exhibited promising inhibitory effect by inducing significant reduction in radial growth and sclerotial formation while *C. procera, C. arvensis* and *A. conzoides* showed moderate growth inhibition. Effective inhibitory action of *D. alba* against *M. phaseolina* has been reported by Shahnaz *et al.* (2010). High level of effectiveness of *D. alba* apparently seems to be linked with antifungal compounds present in plants of *Datura* sp. (Shahwar *et al.,* 1995; Jalander *et al.,* 2012).

CONCLUSION

This investigation concludes that extracts of *D. metel and C. procera* with highest inhibition activity against *M. phaseolina* can play important role in integrated disease management of charcoal rot of sesame plant. However, further research related to the identification of bioactive compounds is necessary for their large scale use.

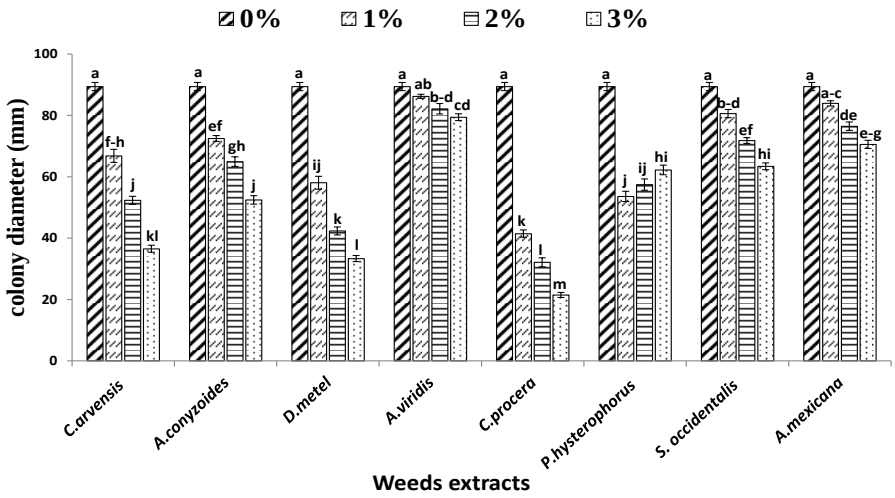

Figure 1. Effect of aqueous extracts of weeds on colony diameter of *M. phaseolina*. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference ($P \leq 0.05$) as determined by DMR test.

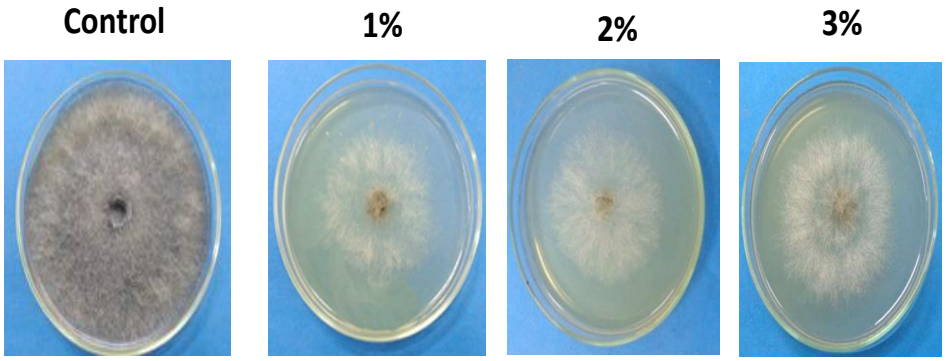

Figure 2. Percentage growth inhibition in radial growth of *M. phaseolina* at different concentration of *P. hysterophorus*

Table-2. *In vitro* effect of different concentration of weed extracts on sclerotia development of *Macrophomina phaseolina*

Treatment	No of Sclerotia				Size of Sclerotia (µm)			
	1%	2%	3%	Highest inhibition %	1%	2%	3%	Highest inhibition %
Convolvulus arvensis	15.6±1.48	12.8±1.08	11.2±1.27	61.33	22.1±1.15	19.4±0.93	12±1.29	57.8
Ageratum conyzoides	14.7±0.97	10.3±0.87	9.4±1.36	70.10	20.6±1.67	15.4+1.23	10.2±0.69	63.8
Calotrpis procera	0	0	0	100	0	0	0	100
Amaranthus viridis	25.9±1.51	23.5±1.25	22.2±1.77	29	26.2±0.86	23.6±1.01	21.6±1.11	23.4
Datura alba	0	0	0	100	0	0	0	100
Parthenium hysterophorus	0	0	0	100	0	0	0	100
Senna occidentalis	21.9±1.07	16.0±1.54	14.4±1.65	53	23.6±0.87	18.6±1.04	13.8±0.99	51
Agremone mexicana	21.1±1.83	19.06±1.65	18.8±1.33	40	25.2±0.76	21.6±1.08	18.6±1.37	34
Control	31.6±0.87	31.6±0.87	31.6±0.87	0	28.2±0.79	28.2±0.79	28.2±0.79	0

Values are mean of three replicates ± standard error

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